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A new synthetic sex attractant for males of *Illiberis* (*Primilliberis*) *pruni* Dyar, 1905 (Lepidoptera: Zygaenidae, Procridinae)

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Abstract

Esters of 2-dodecenoic acid and enantiomers of 2-butanol have been synthesized at the Crimean Federal University. Their attractiveness for Procridinae (Lepidoptera: Zygaenidae) was tested in field observations undertaken in Naruto, Tokushima, Japan in 2014 and 2016. The males of *Illiberis* (*Primilliberis*) *pruni* Dyar, 1905, were attracted to the substance "EFETOV-S-2" (*R*-enantiomer of 2-butyl 2-dodecenoate), while the males of another closely related species *I.* (*P.*) *rotundata* Jordan, 1907, did not react to this substance. The new synthetic sex attractant can be used for detection of orchard pest *I.* (*P.*) *pruni* and monitoring its flight period.
KEY WORDS: Lepidoptera, Zygaenidae, Procridinae, Procridini, *Illiberis pruni*, 2-butyl 2-dodecenoate, pest control, sex attractant, Japan.

Un nuevo atrayente sexual sintético para los machos de *Illiberis* (*Primilliberis*) *pruni* Dyar, 1905 (Lepidoptera: Zygaenidae, Procridinae)

Resumen

Ésteres del ácido 2-dodecenoico y enantiómeros de 2-butanol, han sido sintetizados en la Universidad Federal de Crimea. Su atracción para los Procridinae (Lepidoptera: Zygaenidae) fue evaluado en las observaciones de campo emprendidas en Naruto, Tokushima, Japón en 2014 y 2016. Los machos de *Illiberis* (*Primilliberis*) *pruni* Dyar, 1905, eran atraídos por la sustancia "EFETOV-S-2" (*R*-enantiómero de 2-butil 2-dodecenoato), mientras que los machos de otra especie próxima *I.* (*P.*) *rotundata,* no reaccionaban a esta substancia. El nuevo atrayente sexual sintético, puede ser usado para la detección de la plaga del huerto *I.* (*P.*) *pruni* y monitorizar su período de vuelo.
PALABRAS CLAVE: Lepidoptera, Zygaenidae, Procridinae, Procridini, *Illiberis pruni*, 2-butil 2-dodecenoato, control de plaga, atrayente sexual, Japón.

Introduction

Pheromones are chemical signals that play the central role in communication between members of the same species. Nowadays sex pheromones are used to control agricultural pest moths. High specificity and low toxicity of these molecules have fewer environmental impacts than broad-spectrum pesticides.

According to the modern classification, the family Zygaenidae is divided into five subfamilies: Inouelinae Efetov & Tarmann, 2017; Procridinae Boisduval, 1828 (including tribes Artonini and Procridini); Chalcosiinae Hampson, 1892 (including tribes Chalcosiini, Cyclosiini, Agalopini, Aglaopini and Heteropanini); Callizygaeninae Alberti, 1954; and Zygaeninae Latreille, 1809 (including tribes Pryeriini and Zygaenini) (EFETOV, 1997a, 2001a, 2001b, 2006; YEN, 2003; EFETOV & TARMANN, 2012, 2013b, 2014b, 2016a, 2017b; EFETOV & SAVCHUK, 2013; EFETOV &

KNYAZEV, 2014; EFETOV *et al*., 2014a, 2015a; KNYAZEV *et al*., 2015a, 2015b; MUTANEN *et al*., 2016). The fauna of Japan is represented by Inouelinae, Procridinae, Chalcosiinae and Zygaeninae (INOUE, 1976, 1982; EFETOV, 1997a, 1999; EFETOV *et al*., 2004, 2006; EFETOV & HAYASHI, 2008; EFETOV & TARMANN, 2017a). The structure of sex pheromones and attractants is known only for the representatives of Procridinae (tribe Procridini) and Zygaeninae (tribe Zygaenini) (EFETOV *et al*., 2014b, 2014c, 2015b, 2016; SUBCHEV, 2014; RAZOV *et al*., 2017).

Some Procridinae (Lepidoptera: Zygaenidae) are pest species, for example, *Illiberis* (*Primilliberis*) *rotundata* Jordan, 1907, and *I.* (*P.*) *pruni* Dyar, 1905. These important orchard pests are distributed in Japan (INOUE, 1976, 1982), China, south-eastern Siberia, Far East of Russia (EFETOV, 2005), Korea (KIM *et al*., 2004), and Mongolia (EFETOV *et al*., 2012). *I.* (*P.*) *rotundata* and *I.* (*P.*) *pruni* are found sympatric in cherry, apple, pear gardens as well as ornamental parks. The host-plants of these moths belong to the genera *Cerasus*, *Prunus*, *Malus* and *Pyrus* (Rosaceae) (INOUE, 1976, 1982; EFETOV, 2005).

I. (*P.*) *rotundata* and *I.* (*P.*) *pruni* are two species from the subgenus *Primilliberis* Alberti, 1954, of the genus *Illiberis* Walker, 1854. According to the latest revisions, this genus includes 26 species (EFETOV, 1996, 1997b; EFETOV *et al*., 2004; EFETOV & TARMANN, 2012, 2013a, 2014a, 2016b; MOLLET, 2015, 2016). Twenty-three species were excluded from *Illiberis* s.l. and now represent four other genera: *Pseudoilliberis* Efetov & Tarmann, 2012, *Hedina* Alberti, 1954, *Goe* Hampson, 1893, and *Zama* Herrich-Schäffer, 1885 (EFETOV, 1998, 2010; EFETOV & TARMANN, 2012).

Recently, the main sex pheromone compounds have been identified in extracts of pheromone glands of *I.* (*P.*) *rotundata* female (SUBCHEV *et al*., 2009, 2010). All substances are unsaturated fatty acids esterified with a short-chain chiral alcohol 2-butanol. Further field observations showed that the mixtures of two esters ((2*R*)-butyl (7*Z*)-dodecenoate and (2*R*)-butyl (9*Z*)-tetradecenoate) at ratios of 30:100 - 50:100 were the most attractive for the males of *I.* (*P.*) *rotundata* (SUBCHEV *et al*., 2012). Moreover, the males of closely related species *I.* (*P.*) *pruni* are also attracted by the blend of the same molecules but at other ratios - 100:10 - 100:30 (SUBCHEV *et al*., 2013).

It should be noted that *R*-enantiomers of both esters were attractive for the males of *Illiberis* while *S*-enantiomers were not active. Nevertheless, *S*-enantiomer of 2-butyl (7*Z*)-dodecenoate (alone or in mixtures with *R*-enantiomer) can be attractive for the males of other Procridinae species, viz. *Adscita* (*Adscita*) *geryon* (Hübner, 1813) (SUBCHEV *et al*., 2010; EFETOV *et al*., 2015b), *A.* (*Tarmannita*) *mannii* (Lederer, 1853) (EFETOV *et al*., 2015), *Jordanita* (*Praviela*) *anatolica* (Naufock, 1929) (EFETOV *et al*., 2010) and *J.* (*Rjabovia*) *horni* (Alberti, 1937) (EFETOV *et al*., 2011). Thus, isomerism of pheromone molecules and their combinations are vitally important for males' recognition of conspecific females. In addition, a different ratio of the same sex pheromone compounds can be an isolating mechanism that prevents interbreeding of *I.* (*P.*) *rotundata* and *I.* (*P.*) *pruni* (SUBCHEV *et al*., 2013, 2016).

However, excretion of the natural sex pheromones from special glands, their identification and artificial synthesis are usually expensive and technically difficult processes. Therefore, the search for new attractive molecules for the pest species is of a large practical significance. For this purpose, other esters of 2-butanol and unsaturated acid have been created at our laboratory of the Crimean Federal University. The attractiveness of the synthesized substances for the males of *Illiberis* was estimated during field investigations in Naruto, Tokushima, Japan in 2014 and 2016. The results of this work are reported here.

Materials and methods

The tested attractants have been produced from lauric acid (Ukraine), 2-butanol, (*R*)-(-)-2-butanol (both - Sigma-Aldrich, Germany) as described earlier (EFETOV *et al*., 2014c). The chemical nature of the synthesized 2-butyl 2-dodecenoate was confirmed by the method of nuclear magnetic resonance spectroscopy. The received esters were labeled as EFETOV-2 (racemic mixture of *R*- and *S*-enantiomers of the target ester) and EFETOV-S-2 (*R*-enantiomer).

For preparing lures, attractants were applied onto vial caps composed of grey rubber. The baits were fixed in home-made transparent Delta traps with removable sticky layers (10 cm x 15 cm) covered with insect glue (the Tanglefoot Company, Grand Rapids, Michigan, USA). In all sites that were investigated we also placed control traps without attractant rubber caps (negative control). The traps were hung on trees at a height of 1.0–1.5 m above the ground (Fig. 1).

Figure 1.– Delta trap with attractant EFETOV-S-2 (Campus, 2016) (Photo: C. Koshio).

In 2014 and 2016, we conducted field tests in Naruto, Tokushima in the sites where we had collected *I.* (*P.*) *rotundata* and *I.* (*P.*) *pruni* males applying other attractants in previous years (SUBCHEV *et al.*, 2009, 2012, 2013, 2016). Site A, the Enden-park, is a small cherry garden with *Cerasus* x *yedoensis* (Matsum.) A. Vassil., 1957 (synonym: *Prunus* x *yedoensis* Matsum., 1901) trees. Site B, the Campus of the Naruto University of Education, has *Cerasus speciosa* (Koidz.) H. Ohba, 1992 (synonym: *Prunus speciosa* (Koidz.) Nakai, 1915) trees. Both species of trees are known as the host-plants of *I.* (*P.*) *rotundata* and *I.* (*P.*) *pruni*. These sites correspond to site A and site B in SUBCHEV *et al.* (2009) respectively. The distance between the traps in each site was about 4 m and between the two sites it was 1.340 m.

In 2014, we set traps with attractant EFETOV-2 and control traps in site A. Field tests were being undertaken since 12-VI-2014 to 12-VII-2014 July and often checked during that period.

In 2016, attractant EFETOV-S-2 was used and the field work was organized in site A and site B. The traps with lures and control traps were installed on 21-V-2016 and kept till 6-VII-2016. The traps were checked at intervals of 1 to 4 days and the moths caught were removed after counting their number.

All collected specimens have been determined by examination of the genitalia by K. A. Efetov.

Results and discussion

I. (*P.*) *rotundata* occurs every year in all investigated sites. We found some adults including copulating pairs near the traps baited with our attractants, but the males were not found in the traps in 2014 nor in 2016.

In 2014, EFETOV-2 did not attract the males of *I.* (*P.*) *pruni* either. However, the males of this species were caught in traps baited with EFETOV-S-2 in 2016 (Fig. 2). It should be noted that neither *I.* (*P.*) *pruni* larvae nor cocoons were found in studied sites.

Figure 2.– Attracted male of *I.* (*P.*) *pruni* on sticky layer in trap baited with EFETOV-S-2 (Photo: C. Koshio).

The dates of inspection of the traps in different biotopes and quantities of *I.* (*P.*) *pruni* specimens glued in traps are listed below.

Site A: the Enden-park (West), Naruto, Tokushima, Japan: 2 ♂♂, 4-VI-2016; 4 ♂♂, 9-VI-2016; 2 ♂♂, 10-VI-2016; 1 ♂, 14-VI-2016; the Enden-park (East), Naruto, Tokushima, Japan: 3 ♂♂, 9-VI-2016; 2 ♂♂, 10-VI-2016.

Site B: the Campus, Naruto, Tokushima, Japan: 2 ♂♂, 8-VI-2016; 4 ♂♂, 9-VI-2016; 2 ♂♂, 10-VI-2016; 1 ♂, 14-VI-2016.

The control traps (without attractant) were empty in all cases.

Totally, twenty-three males of *I.* (*P.*) *pruni* were attracted to (2*R*)-butyl 2-dodecenoate, whereas *I.* (*P.*) *rotundata* males were absent in traps. A maximum of four *I.* (*P.*) *pruni* males were captured in a single trap during one day trapping period. The seasonal flight of *I.* (*P.*) *pruni* males is shown in Fig. 3.

As it was shown earlier, the synthetic racemic 2-butyl 2-dodecenoate attracted the males of many Procridinae species: *Theresimima ampellophaga* (Bayle-Barelle, 1808) (EFETOV *et al*., 2014c), *Rhagades* (*Rhagades*) *pruni* ([Denis & Schiffermüller], 1775), *A.* (*A.*) *geryon*, *J.* (*Tremewania*) *notata* (Zeller, 1847), *J.* (*Jordanita*) *graeca* (Jordan, 1907), *J.* (*J.*) *globulariae* (Hübner, 1793), and *J.* (*Solaniterna*) *subsolana* (Staudinger, 1862) (EFETOV *et al*., 2016; CAN CENGIZ *et al*., 2018). However, according to our field works in Japan in 2014 this attractant was inactive for *I.* (*P.*) *rotundata* and *I.* (*P.*) *pruni*. Despite the fact that the traps with EFETOV-2 (2-butyl 2-dodecenoate) were placed during the flight period of both species, no specimens were caught in 2014.

On the other hand, EFETOV-S-2 (*R*-enantiomer of 2-butyl 2-dodecenoate alone) was attractive only for the males of *I.* (*P.*) *pruni*, but not for the males of *I.* (*P.*) *rotundata*. These results correspond well with the fact that the males of *I.* (*P.*) *pruni* were also attracted by *R*-enantiomers of the esters of other acids (SUBCHEV *et al.*, 2013, 2016).

It is well known that different moth species have different reactions to the mixtures of attractants

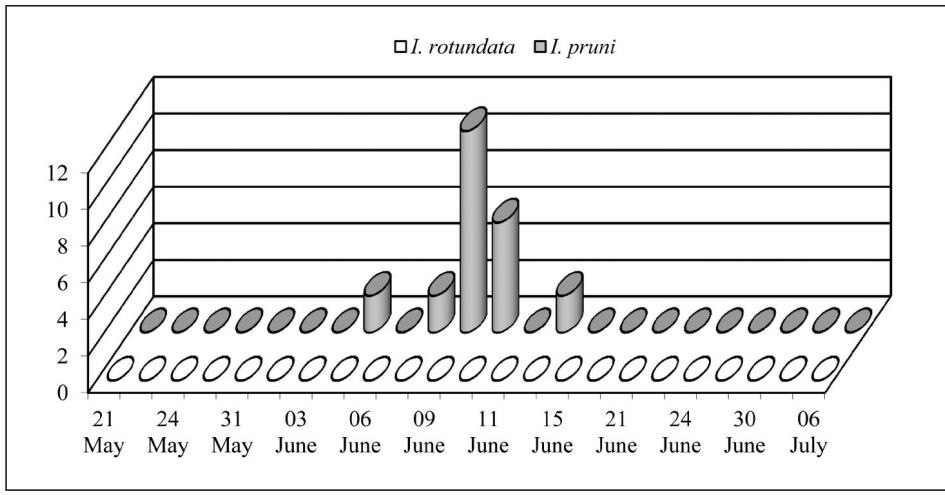

Figure 3.– Seasonal flight of *I.* (*P.*) *pruni* males established by traps with EFETOV-S-2 in Naruto, Tokushima, 2016. No one *I.* (*P.*) *rotundata* male was attracted. *Illiberis* males were absent in the control traps in all sites.

(EFETOV *et al*., 2011, 2014b, 2015b, 2016; SUBCHEV *et al.*, 2013). In the case with *I.* (*P.*) *pruni* it is clear that the presence of (2*S*)-butyl 2-dodecenoate inhibits the attractiveness of (2*R*)-butyl 2-dodecenoate. The males of another closely related species, viz. *I.* (*P.*) *rotundata*, do not react to (2*R*)-butyl 2-dodecenoate (alone or in mixture with (2*S*)-butyl 2-dodecenoate).

Thus, moth males can distinguish chemically similar compounds while searching for females. This should be taken into account when producing synthetic lures, especially for the pest species.

Conclusion

The males of *Illiberis* (*Primilliberis*) *pruni* were attracted to the substance "EFETOV-S-2" (*R*-enantiomer of 2-butyl 2-dodecenoate), while the males of another closely related species *I.* (*P.*) *rotundata* did not react to this substance. The new synthetic sex attractant can be used for detection of orchard pest *I.* (*P.*) *pruni* and monitoring its flight period.

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